

Inversion of the circadian rhythm of melatonin in the Smith-Magenis syndrome

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Objective: The objective was to determine the circadian rhythm of melatonin in the Smith-Magenis syndrome (SMS), which causes behavioral problems and sleep disturbance.

Study design: Questionnaires, sleep consultations, and sleep diaries were obtained in 20 children with SMS (9 girls, 11 boys aged 4 to 17 years). Actigraphy, electroencephalography, and the circadian variations of plasma melatonin, cortisol, and growth hormone were recorded in 8 patients. Early sleep onset, early sleep offset, and sleep attack indicated sleep disturbance.

Results: All children with SMS had a phase shift of their circadian rhythm of melatonin. Time at onset of melatonin secretion was 6 AM \pm 2 (control group: 9 P.M. \pm 2). Peak time was 12 PM \pm 1 (control group: 3:30 AM \pm 1:30), and melatonin offset was at 8 PM \pm 1 (control group: 6 AM \pm 1). Behavioral problems correlated with the inverted circadian rhythm of melatonin.

Conclusion: Considering that clock genes mediate the generation of circadian rhythms, we suggest that haploinsufficiency for a circadian system gene mapping to chromosome 17p11.2 may cause the inversion of the circadian rhythm of melatonin in SMS. (*J Pediatr* 2001;139:111-6)

Smith-Magenis Syndrome is a contiguous gene syndrome^{1,2} ascribed to interstitial deletions of chromosome 17p11.2.³ Clinical features include craniofacial anomalies, short stature, brachydactyly, developmental delay,

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and abnormal behavior.⁴ Behavioral problems include hyperactivity, attention deficit, self-injury, tantrums, and sleep disturbance.^{5,6} Because sleep disturbance has a major impact on patients with SMS and their families, we studied this symptom in 20 children with SMS. All subjects had very uncommon sleep habits. Indeed, they went to bed early (at 8 or 8:30 PM), had 1 to 3 arousals per night, and consistently woke up between 4 and 6 AM. The children felt tired in the morning and had naps during the day and frequent temper tantrums and "sleep attacks" in the evening, that is, they suddenly fell asleep during dinner. These features suggested that an alteration of circadian rhythms could trig-

ger sleep disturbance and cause advanced sleep phase in SMS, especially because an abnormal rhythm of 6 sulfatoxymelatonin, the main urinary melatonin metabolite, has been previously reported by Potocki et al⁷ in SMS. For this reason we have investigated plasma melatonin secretion. Here we add to the view that the circadian rhythm of melatonin is disturbed in SMS.

SMS Smith-Magenis Syndrome

PATIENTS AND METHODS

A total of 20 children with SMS (9 girls, 11 boys) aged 4 to 17 years were included in the study. Inclusion criteria were (1) typical behavioral and dysmorphic features and (2) cytogenetic and fluorescence in situ hybridization analysis evidence of chromosome 17p11.2 deletion with an ONCOR probe (D17S258). Questionnaires and sleep diaries were sent to parents and filled out at home over a period of 1 month. The age-matched control group consisted of children and adolescents recruited in a healthy pediatric population (questionnaires: n = 30). The same investigator (H.D.L.) performed all sleep consultations. For ambulatory actigraphy (8 patients), recordings were made during 1-minute periods with the Actiwatch-score (Cambridge Neurotechnology). Outpatient acti-

Table. Sleep parameters in Smith-Magenis syndrome (n = 20)

No.	Sex	Age (y)	Bedtime	Night sleep		Awakenings (n)	Wake-up time	Naps >30 min		Tiredness (present +, absent-)	Sleep attacks	
				Control	(h)			Control	(n)			
1	M	4	9:00 PM	4-6 y	8	4-6 y	2	6:00 AM	4-6 y	1	+	+
2	M	4	8:00 PM		7		1	4:00 AM		1	+	+
3	F	4	8:30 PM	n = 10	8	n = 10	3	6:00 AM	n = 10	2	+	+
4	F	5	8:00 PM		7		1	6:00 AM		3	+	+
5	F	5	8:00 PM	7:30-9 PM	8	10-11	2	4:30 AM	7-8:30 AM	2	+	+
6	F	6	8:30 PM		9		2	5:00 AM		3	+	+
7	F	7	8:30 PM		9		1	6:30 PM		1	+	+
8	F	7	8:00 PM	7-13 y	7	7-13 y	3	6:30 PM	7-13 y	1	+	+
9	M	8	9:00 PM		8		2	5:30 AM		2	-	+
10	M	9	8:00 PM	n = 10	9	n = 10	2	6:00 AM	n = 10	1	+	+
11	M	10	8:30 PM		6		3	5:30 AM		1	-	+
12	F	10	8:00 PM	8:30-10 PM	8	8:30-10	3	5:00 AM	7-8 AM	1	+	+
13	F	12	8:30 PM		8		2	5:00 AM		1	+	+
14	M	13	9:00 PM		7		1	4:30 AM		2	-	+
15	M	13	9:00 PM		8		1	6:00 AM		1	+	+
16	F	14	8:00 PM	14-17 y	5	14-17 y	3	4:00 AM	14-17 y	1	+	+
17	F	14	8:30 PM		8		2	5:00 AM		1	+	+
18	F	16	10:00 PM	n = 10	6	n = 10	2	6:00 AM	n = 10	1	+	+
19	M	17	8:30 PM	9:30-11:30 PM	7	7-9:30	1	4:30 AM	7-8:30 AM	1	+	+
20	M	17	8:30 PM		8		2	4:30 AM		1	+	+
Mean		9.5	8:30 PM		7.30 h		1,65	5:30 AM		1,5	79%	100%
Range		4-7	8:00-10:00 PM		5-9		1-3	4:00-6:30 AM		1-3		

graphy data over 8 to 14 days were collected, with simultaneous sleep diaries on which parents recorded the sleep and wake times. Activity amplitude was determined by the number of switch closure during each measurement. Actigraph data were analyzed for average activity offset and onset with the Actiwatch software programs.

Eight children with SMS (5 boys, 3 girls aged 4 to 17 years) were admitted for 24 hours between April and June 1999 in a pediatric unit for Medilog 9000 electroencephalography recording (including 6 electroencephalography channels, 1 echocardiography channel, and 1 electromyography channel), plasma and urine melatonin, and cortisol and growth hormone monitoring after approval of our ethical committee and an informed consent of the parents were obtained. None of the children were given melatonin or other drugs for the 2 weeks before the study. Blood samples were drawn from an indwelling forearm catheter hourly, from 8 PM to 8 AM, and at 2-hour intervals from 8 AM to 6 PM. Samples were transferred to heparinized plastic tubes, centrifuged, and frozen at -20° . The children's spontaneously voided urine was collected, the total volume and time of each sample were recorded, and 10-mL aliquots were frozen. Melatonin and 6 sulfatoxymelatonin, the main urinary melatonin metabolite, were measured by radioimmunoassay (mean sensitivity of 5 pg/mL).^{8,9} The internal coefficient of variation for the melatonin assay was routinely <7% (between 30 and 200 pg/mL). The aged-matched control group (15 subjects) consisted of healthy children and adolescents hospitalized for idiopathic small stature.

RESULTS

Questionnaires, sleep consultations, and sleep diaries revealed sleep disturbance in all 20 subjects with SMS. These symptoms had a major impact on the children with SMS and their families. All children went to bed easily after a short bedtime ritual. Bedtime was similar at 8 to 9 PM regardless of age and sex (4 through 17 years, control group aged <6 years = 7:30 to 9 PM, control group aged 7 to 13 years = 8:30 to 10 PM, control group aged 14 to 17 years = 9:30 to 11:30 PM; see Table). The duration of night sleep averaged 7.30 hours (range, 5 to 9 hours), declined with age, and was slightly shorter than that of the age-matched control group (control group <6 years = 10 to 11 hours, control group aged 7 to 13 years = 8.30 to 10 hours, control group aged 14 to 17

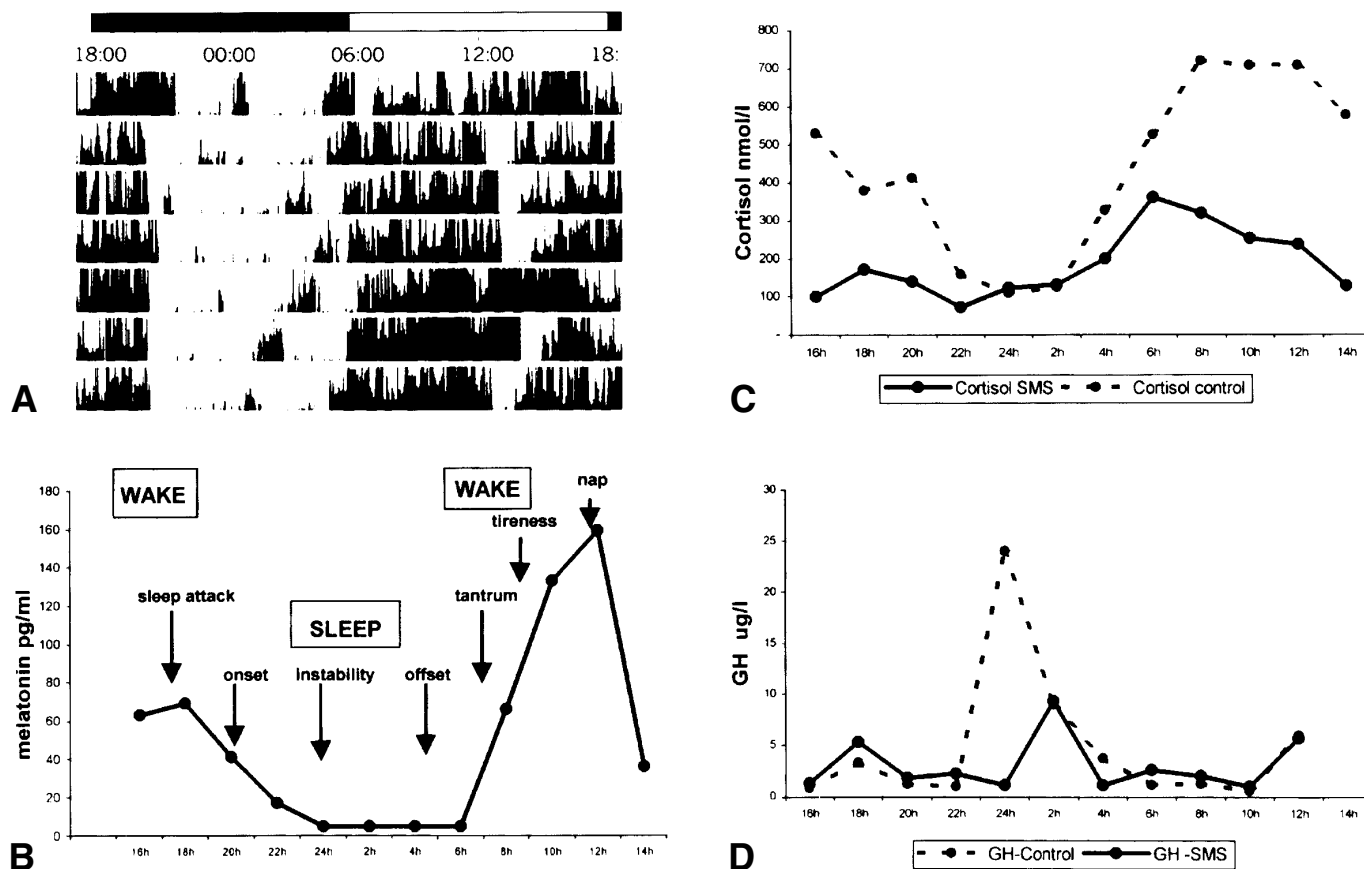


Fig 1. Twenty-four-hour actigraphy, day-night behavior, and 24-hour profiles of plasma melatonin, growth hormone, and cortisol in Smith-Magenis syndrome. **A**, Twenty-four hour actigraphy of 9-year-old child with SMS. Filled bars indicate periods of movements and activity. **B**, Day and sleep behavior in same child with SMS. **C-D**, Twenty-four-hour profiles of plasma cortisol and growth hormone in 9-year-old child with SMS compared with age-matched control. Growth hormone values are lower than those of control group, but secretion is protracted and total amount of secreted growth hormone falls within normal range. Solid lines, SMS group. Dotted lines, control group.

years = 7 to 9.30 hours). The children consistently woke up 1 to 3 times per night and fell back asleep within 30 minutes. Once awakened, they were hyperactive. This behavior forced parents to look after the child constantly and to devise methods to keep him in the bedroom at night (lock the door; switch light; remove small, dangerous objects). The mean wake-up time was 5.30 AM (range 4 to 6:30 AM; control group <6 years = 7 to 8:30 AM, control group aged 7 to 13 years = 7 to 8 AM, control group aged 14 to 17 years = 7 to 8:30 AM). Behavioral problems correlated with night sleep insufficiency. Most patients (79%) exhibited morning tiredness, when circadian vigilance is high in unaffected children. They had temper tantrums when tired

(65%) and naps (more than 30 minutes) during the day until 17 years. Most interestingly, they consistently had “sleep attacks,” that is, they suddenly fell asleep during evening meals.

Actimetry in the 8 hospitalized children correlated with sleep diaries and confirmed instability of sleep, frequent microarousals, and naps during the day (Fig 1). The 24-hour polysomnography revealed a reduced total sleep time (not shown). All sleep states were present, but 3 to 4 nonrapid eye movement sleep was reduced. Rapid eye movement sleep was present but disrupted in all children, and arousals with increased tonic electromyographic activity were frequent. Awakenings (>15 minutes) occurred in 75% of cases (not shown).

It is interesting that all children with SMS tested displayed a phase shift in their circadian rhythm of melatonin (Fig 2). Indeed, time at onset of melatonin secretion in SMS was 6 AM \pm 2 (control group: 9 PM \pm 2, n = 15). Peak time was 12 PM \pm 1 (control group: 3:30 AM \pm 1:30, n = 15), and melatonin offset was at 8 PM \pm 1 (control group: 6 AM \pm 1, n = 15). Irregular levels of melatonin were noted during the day, with a second peak between 6 and 8 PM (45 pg/mL \pm 32, n = 8), and the total duration of melatonin secretion was protracted in SMS (15.5 hours \pm 3.5, n = 8; control group: 8 hours \pm 1, n = 15; peak value = 94 \pm pg/mL, n = 8; control group: 76 pg/mL, n = 15). In a similar fashion, urinary melatonin and 6-sulfatoxymelatonin revealed an in-

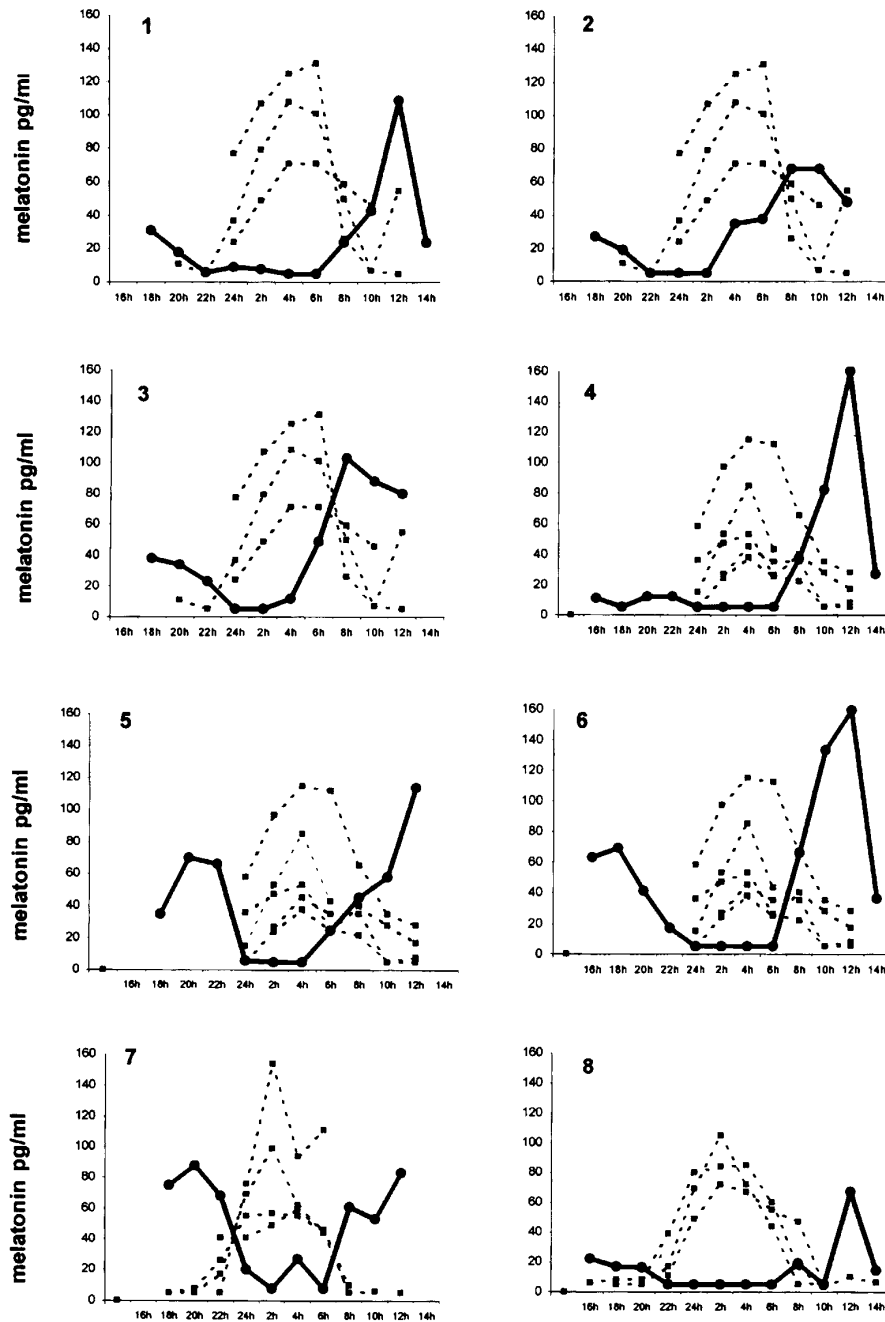


Fig 2. Circadian variation of plasma melatonin in children with SMS and control group. *Solid lines*, children with SMS aged 5 to 6 years (panels 1-3), 7 to 8 years (panels 4-6), 12 years (panel 7), and 17 years (panel 8). *Dotted lines*, age-matched control group; 5 to 6 years (3 controls), 7 to 9 years (5 controls), 12 years (4 controls), and 17 years (3 controls). Aged-matched control group included healthy children or adolescents hospitalized for small stature, with normal results of cortisol, growth hormone, and melatonin values.

verted night/day ratio in all 8 children with SMS (not shown).

Cortisol followed the usual circadian secretion and was in the normal range (peak value = 217.2 ± 12.1 nmol/L, n = 8; control group: 184 ± 38 nmol/L, n =

15; time of secretion = 7:30 to 9:50 AM, n = 8; control group: 7:05 to 11:15 AM, n = 15; Fig 1). Growth hormone was in the normal range (peak value = 9.50 ng/mL, n = 8; control group: 24 ng/mL, n = 15; peak time = 10:50 PM, n = 8;

control group: 11:15 PM, n = 15). The values were lower than in the control group, but the duration of secretion was protracted, and the total amount of secreted growth hormone fell within normal ranges.

DISCUSSION

This study reports severe sleep disturbance and phase shift of the circadian rhythm of melatonin in a series of children with SMS. Similar findings on disturbed sleep pattern and urinary excretion of 6 sulphatoxymelatonin have been reported.¹⁰ Early sleep onset, frequent awakenings, and early sleep offset were consistent features of the disease and are highly specific diagnostic criteria in SMS. "Sleep attacks" occurring at the end of the day may represent the endogenous sleep onset of the children that therefore could be regarded as equivalent to a sleep phase advance. According to this hypothesis, the endogenous sleep onset time of the children would be masked by the imposed social activities.

Sleep disturbances had a major impact on children with SMS and their families. We could correlate tantrums with melatonin rise. Naps and sleep attacks occurred when melatonin peaked at midday and in the evening, respectively (Fig 1). Considering that behavioral problems correlate with the inverted circadian rhythm of melatonin in SMS, we hypothesize that at least part of the hyperactivity and attention deficit occurred because the patients struggled against sleep, when melatonin rose during the day. Yet it is difficult to decide whether sleep disturbance is caused by the abnormal melatonin rhythm or perhaps both abnormalities (sleep and melatonin) are caused by an intrinsic disorder of the circadian clock. Our data are fully consistent with the sleep cycle parameters reported by Smith et al⁶ in SMS. Indeed, although it has been estimated

that 10% to 30% of otherwise healthy children have sleep disorders,¹¹ 100% of children with SMS have multiple sleep problems. Sleep patterns in children with mental retardation and severe behavior disorders display significantly less total sleep than their peers of the same age, and 88% of them had disturbances of sleep, namely, delays in getting to sleep, frequent night waking, or early waking.^{12,13} However, the complete typical sleep pattern in SMS (early sleep onset, awakenings, early sleep offset) is unusual among sleep disturbances in children with neurodevelopmental disability.

Melatonin, the main hormone of the pineal gland, is synthesized from serotonin.^{14,15} Its synthesis and release are stimulated by darkness and inhibited by light. Light or dark entrainment proceed through the retino-hypothalamic tract to reach the suprachiasmatic nuclei of the anterior hypothalamus.¹⁶ Suprachiasmatic nuclei contain biologic clocks, which are endogenous pacemakers generating circadian rhythms entrained by environmental stimuli (eg, light). A number of clock genes controlling circadian rhythms have been recently identified in higher eukaryotes. Their expression shares common features across species. It oscillates with a 24-hour rhythm and persists in the absence of environmental cues. It is reset by changes in light/dark cycle and undergoes negative feedback that downregulates activity.^{17,18} Considering that clock genes are expressed in a circadian pattern in suprachiasmatic nuclei, one can hypothesize that haploinsufficiency for a clock gene could account for sleep disturbance in SMS. It is interesting that subunit 3 of the COP9 signal transduction complex (*COPS3*) maps within the SMS critical region in 17p11.2.¹⁹ COP9 is essential for the light control of gene expression during plant development and is conserved across species. It has been recently shown that the gene for subunit 3 of the COP9 signal transduction com-

plex, *COPS3*, is expressed in transformed lymphoblastoid cell lines of patients with SMS,²⁰ with no obvious difference in the amount of *COPS3* protein between patients and parental controls.¹⁰

Circadian rhythmicity not only involves clock genes but also requires (1) an input signaling pathway for detection of exogenous signals and their transmission to suprachiasmatic nuclei by the retino-hypothalamus and (2) an output signaling pathway of postganglionic fibers ascending to the pineal gland to maintain melatonin secretion under the control of the suprachiasmatic nuclei.¹⁴ Consequently, the inversion of the circadian rhythm of melatonin in SMS may also result from an alteration of the input/output-signaling pathway (eg, photic entrainment in the retina/retino-hypothalamic or β 1-adrenergic signaling transduction to the pineal gland).

How melatonin acts on sleep is presently unknown. Melatonin may modify brain levels of monoamine transmitters, thereby initiating a cascade of events culminating in the activation of sleep mechanisms, probably by inhibiting the wakefulness structures.^{21,22}

In conclusion, this study gives strong support to the view that the circadian rhythm of melatonin is shifted in SMS and suggests a disturbed circadian behavior in this disease. These observations are particularly relevant to therapeutic approaches in SMS. Indeed, melatonin administration is not necessarily warranted, because the amount of secreted hormone is largely normal but its kinetic is erratic. Future therapeutic approaches may include (1) blockade of endogenous melatonin signaling pathways combined with on-time exogenous melatonin administration^{23,24} or (2) light therapy.²⁵

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